requirement of membrane depolarization and intracellular Ca^{2+} increase for channel activation. We recently developed a novel cell-based assay system utilizing a hyperactive mutant BK_{Ca} channel. The mutant channel was generated by introducing two point-mutations into the cytosolic flexible interface between the two RCK domains of the wild-type BK_{Ca} channel. The mutant channel exhibited a large negative shift in its conductance-voltage relationship, which indicates activation by modest depolarization at resting concentrations of intracellular Ca^{2+}. When tested in a commercially available K^+ channel assay, cell-lines stably expressing the hyperactive BK_{Ca} channel generated a strong fluorescence signal under conditions that are typical for voltage-gated K^+ channels. By screening over 2,000 compounds in the libraries of chemical and natural products, we were able to obtain several new BK_{Ca} channel modulators either potentiating or inhibiting the channel activity. We are currently investigating the mechanism of action for these compounds using electrophysiological means. Thus, our new cell-based assay platform can be used efficiently to screen novel modulators of BK_{Ca} channels in a high-throughput manner.

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3727-Pos Board B455

Double Electron-Electron Resonance Studies of Ligand Induced Rearrangements of HCN Channels

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Hyperpolarization-activated cyclic nucleotide-modulated (HCN) ion channels are members of the superfamily of voltage-gated potassium channels. HCN channels play a role in controlling neuronal and cardiac pacemaking activity. They are regulated by the binding of cyclic nucleotides to a conserved, intracellular cyclic nucleotide-binding domain (CNBD). The CNBD is connected to the transmembrane domain through a C-linker. Crystallization studies of intracellular fragments of the C-linker and CNBD show little conformational difference in cAMP bound and unbound states. However, fluorescence and electrophysiology experiments suggest a significant conformational change in the C-linker and CNBD. Here, we use double electron-electron resonance (DEER) to study conformational changes of a soluble fragment of HCN channels in response to binding different cyclic nucleotide species. DEER is an electron paramagnetic resonance technique capable of measuring absolute distances and distance distributions between two spin-labeled protein residues separated by 2 to 8 nanometers. The ability to measure distance distributions allows us to probe whether binding of a partial agonist induces a conformational change smaller compared to the full agonist or results only in a shift of the conformational equilibrium. We find that in a soluble fragment of HCN2 consisting of the C-linker and CNBD, cAMP, the physiological agonist, leads to conformational changes of the CNBD that are much greater than suggested by crystallization. Binding of cCMP, known to be a partial agonist of the full channel, leads to conformational changes similar to cAMP. We also find that cGMP, known to be a full agonist on intact channels, acts as a partial agonist on the fragment, causing only a fraction of protein to undergo conformational change.

3728-Pos Board B456

The Molecular Basis of KCNH Channel Regulation by the EAG Domain

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The KCNH voltage dependent potassium channels are key regulators of cellular excitability, involved in cardiac long QT syndrome type 2 (LQTS2), epilepsy, schizophrenia and cancer. The intracellular domains of KCNH channels are structurally distinct from other voltage-gated channels. The amino-terminal region contains an eag domain, which includes a Per-Arnt-Sim (PAS) module and a PAS-cap region, while the carboxy-terminal region encompasses a cyclic nucleotide-binding homology domain (CNBHD), connected to the pore domain through a C-linker domain. These specialized intracellular domains are the site of many disease-causing mutations and bestow unique gating and regulation on KCNH channels. It has been suggested that the eag domain may interact with either the S4-S5 linker or the CNBHD in human ERG (hERG) and EAG channels. We have used fluorescence approaches to determine that the eag domain and the CNBHD, from the mEAG1 channel, form a complex in solution, with an apparent affinity of 13.2 ± 2.3 μM. Moreover, an equimolar mixture of purified eag domain and CNBHD produced co-crystals of the complex, belonging to the P65 space group, which diffracted to 2.0 Å resolution. The structure of the eag domain-CNBHD complex was solved using molecular replacement with mEAG1 CNBHD as a model. Harboring many LQTS2 and cancer-associated mutations, the eag domain-CNBHD interface involves three important regions: (i) the "intrinsic ligand" motif, a unique structural feature of the CNBHD; (ii) the post-CNBHD region, known to mediate EAG channels regulation by a variety of cellular signaling events; and finally, (iii) the PAS-cap region, which constitutes the first 25 amino acids of the eag domain, and forms a highly conserved amphipathic helix (αCAP). The structure of the EAG domain-CNBHD complex of mEAG1 provides a detailed physiological and pathophysiological description of the intracellular domain of the KCNH channels.

3729-Pos Board B457

Rosetta Structural Modeling of Tarantula Toxin Binding to Voltage Sensors

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Arachnids produce inhibitory cystine knot (ICK) peptide toxins that are potent allosteric modulators of ion channel voltage sensors. This study investigates the structural basis of voltage sensor toxin interaction. Guangxitoxin-1E (GxTX) and Scodra griseipes toxin (SgTX) inhibit Kv2.1 channels by binding to the third segment of the voltage sensor (S3b), but their sequences share only the cystine residues required for their folding motif. Using Rosetta structural modeling we constructed theoretical complexes of Kv2.1 with GxTX and SgTX. These toxins were docked to the α-helical tarantula toxin receptor site on the S3b region of the activated Kv2.1 voltage sensor paddle. These amphiphilic toxins partition into lipid bilayers and modulate channels by interacting with extracellular solution and membrane lipids as well as their receptor site. To better understand the importance of solution and lipid contacts, docking simulations were preformed in both aqueous and membrane-like environments. In aqueous environments, the complexes identified energetically favorable interfaces with the receptor site. Toxin docking with an implicit membrane yielded convergent structures with SgTX, but similar complexes with GxTX did not find energetic minima. The interaction surfaces in these membrane embedded models of SgTX compare favorably with key residues identified by experimental alanine scans. Voltage clamp recordings and fluorescent measurements of toxin binding to Kv2.1 reveal that GxTX has a greatly weakened affinity for activated voltage sensors. Our results are consistent with GxTX having a lower affinity for activated voltage sensors than SgTX. We propose the binding site for GxTX, but not SgTX, is occluded when voltage sensors are activated.

3730-Pos Board B458

Common Interaction Surfaces for Tarantula Toxins Targeting Kv and ASIC Channels

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Gating-modifier tarantula toxins, such as hanatoxin and GxTx-1E, have been shown to partition into membranes to interact with voltage-sensors of voltage-gated ion channels. While relatively little is known about the specific molecular interactions between these toxins and voltage sensors, PcTx1 is a related gating-modifier tarantula toxin that was recently crystallized in complex with acid sensing ion channels (ASIC); demonstrating that the toxin binds to the extracellular domain, where it interacts with helix-5 and inserts an Arg-finger into the subunit interface. Although GxTx-1E and PcTx1 interact with structurally unrelated ion channels in different environments (membrane vs solution), their structures are remarkably similar (backbone RMSD~3 Å). To compare the binding surfaces of GxTx-1E and PcTx1, we carried out Ala scanning mutagenesis on GxTx-1E and studied the interaction of each mutant with the Kv2.1 channel using electrophysiology. Our results identify an active surface of GxTx-1E that overlaps extensively with the binding surface of PcTx1 for ASIC. To further compare toxin binding surfaces, we constructed channel chimeras in which helix-5 from ASIC was transferred into Kv2.1, and a toxin chimera, in which two critical loops of GxTx-1E were